MICROORGANISMS IN SEED DECAY

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MICROORGANISMS IN SEED DECAY

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A B S T R A C T

This study was undertaken to compare the tendency of wheat, flax and pea seed to decay in the soil, to determine the ability of the seed-decay organisms encountered to act under different conditions, and to ascertain the relative persistence of different chemical seed protectants on these seeds in the soil.

Under all soil environmental conditions tested, pea seed showed the greatest tendency to decay, wheat the least, while flax was intermediate. The majority of the seed decay organisms were active over a fairly wide range of soil temperature and moisture conditions. Many of the fungal isolates were capable of causing seed decay of both wheat and flax in the soil, but not of peas.

Orthocide 406 was the only fungicide which persisted on wheat, flax or pea seed for more than two days in the soil. Except for this fungicide, the mercurial preparations persisted longer than the non-mercurial preparations on these seed types in the soil. The majority of the six fungicides tested were more persistent on wheat and pea seed than on flax seed in the soil.

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MICROORGANISMS IN SEED DECAY

INTRODUCTION

Each year large quantities of seeds are sown, of which many fail to produce plants. Microorganisms causing seed decay are largely responsible for seedling mortality. Severity of such damage is determined by several factors, among which, quantity of inoculum, host susceptibility, soil temperature and moisture, are among the most important. In general, the soil inhabiting organisms which cause seedling blights, usually become aggressive when the environment is unfavorable for the growth of the seedling.

In the prevention of seed decay, one must endeavor to render the environment unfavorable for the causal microorganisms, and favorable for the host. Chemicals may be used to advantage, but only those which combine germicidal and non-phytotoxic properties with low cost, are likely to be used.

OBJECTS OF THE INVESTIGATION

This investigation is centered around three distinctly different types of seed, wheat, flax and peas; the object being to determine:

(a) their relative susceptibility to decay by the common soil microflora under different soil environmental conditions;

(b) the types of microorganisms responsible for their decay;

(c) the effect of different factors, including soil moisture and temperature, upon the activity of microorganisms responsible for seed decay;

(d) the ability of the microorganisms that rot one type of seed to rot the other two types of seed;

(e) the relative persistence of commercial seed protectants on seeds in the soil.

EFFECT OF ENVIRONMENTAL CONDITIONS ON EMERGENCE

INTRODUCTION

It is known that seedlings as well as microorganisms respond differently to different soil environmental conditions. The object of the following experiments was therefore to determine the influence of two soil environmental factors, the soil moisture and the soil temperature at which wheat, flax and pea seed are most, and least, susceptible to decay by the common soil microflora. Dickson (9) found that wheat and corn seedlings were only predisposed to seedling blight when they were grown at temperatures unfavorable to their growth. Wilson (44, 45) studied the rate and amount of germination and its relation to mold attack on wheat, on blocks of plaster of Paris. The effect of soil moisture and temperature on pea emergence has been studied by Ledingham (23), Baylis (4), Hull (15) and Wallace (40); Baylis (3) gave an account of the fungi concerned.

METHODS

The tendency of wheat, flax and pea seed to decay was compared by exposing these seeds in non-sterile soil to various moisture and temperature conditions during their

germination and emergence. The amount of decay was calculated by comparing the emergence of untreated seed with that of seed treated with Ceresan M. Ceresan M is one of the best known organic mercurial seed protectants. Ceresan M was applied in dust form at the rate of ½, 1 and 1 oz. per bushel for wheat, flax and peas, respectively. The seeds used were 1949 Red Bobs wheat, 1948 Redwing flax and Homesteader peas. These seeds germinated 95 percent and over in a blotter test.

Non-sterile, 3:1 Edmonton soil-sand mixture was used in all the experiments. Edmonton soil is described as a heavy clay; the soil used in these experiments was high in organic matter. The seeds were sown at a depth of 1 inch. All the pot experiments were carried out in the greenhouse in which the air temperature was maintained at 15°C. Significant differences in emergence were calculated by analysis of variance.

EFFECT OF DIFFERENT LEVELS OF SOIL MOISTURE ON EMERGENCE

The moisture experiments were carried out in 5½ inch pots, using 4 replicates and 25 seeds per pot. The moisture content of the soil was determined every 2 or 3 days and maintained at the designated level. The results are presented in Table I.

TABLE I

EFFECT OF DIFFERENT LEVELS OF SOIL MOISTURE ON THE EMERGENCE OF WHEAT, FLAX AND PEAS IN SOIL

Treatment	Number of Seedlings Emerged		
	Low Soil Moisture	Optimum Soil Moisture	High Soil Moisture
Wheat	15.5$_{xx}$	22.5	21.5
Wheat Ceresan M $\frac{1}{2}$ oz.	23.0	23.5	22.3
Flax	8.0	8.8$_{xx}$	9.5$_{xx}$
Flax Ceresan M 1 oz.	12.0	15.5	17.5
Peas	1.8	1.5	0.0$_{x}$
Peas Ceresan M 1 oz.	5.3	4.0	3.8

x significant between treated and untreated
xx very significant between treated and untreated

When a difference is referred to as being significant, it means that a difference as great would occur 5 or less times out of 100 by chance alone, and if highly significant, a difference as great would only occur 1 or less times out of 100 by chance alone.

Under low, optimum and high soil moisture conditions, peas showed the greatest tendency to decay, flax the next greatest tendency, and wheat the least tendency to decay. Wheat and flax were more subject to decay at low soil moisture, while peas were more subject to decay at high soil moisture. Ceresan M significantly increased the emergence over non-treated seed to the 1 percent level in wheat under low soil moisture conditions, and flax under optimum and high soil moisture conditions. The emergence of peas was only significantly increased to the 5 percent level under high soil

moisture conditions.

Discussion

These results are very similar to those obtained by other workers. Wilson (44) found that wheat showed a wide adaptation to varying moisture conditions. A soil moisture content of 50 percent of saturation resulted in high germination with all the spring and winter wheat varieties he studied.

That flax when treated with Ceresan M should give the highest emergence in high soil moisture and the lowest in low soil moisture, suggests that soil moisture may play a part in fixing the mercurial dust to the seed. Muskett (36) found that the effectiveness of mercurial dusts is related to their ability to adhere to the seed, by obtaining better results when the dusts are applied and afterwards fixed to the flax seed by the use of water or separated milk.

The relatively low emergence in the peas was probably due to watering them immediately after sowing. Baylis (4) found that watering peas immediately after sowing markedly depressed emergence, and a steady improvement was shown as the date of watering was postponed. A safe practice is to sow the seed in slightly moistened soil and not to water it until at least three days after sowing. There appears to be a critical period during the course of germination when high soil moisture is very deleterious. The duration of this

period depends upon the rate of germination, which is related to soil temperature. Baylis (3) stated that the most important fungi concerned in preemergence blight of peas are Pythium and Fusarium species. Hull (15) concluded that the severity of preemergence blight of peas is greatly accentuated by high soil moisture and that a certain measure of control could be obtained by dusting the seed with a commercial mercurial preparation. He found that the mercurial compound resulted in the greatest increase in stand when the soil was wet and when wrinkled-seeded peas were used. On the other hand, there is a risk of phytocidal damage when the treatments are applied under dry soil conditions. It was also found in the present studies that only under high soil moisture conditions was a significant difference between the emergence of treated and untreated pea seed obtained.

Summary

Under all the soil moisture conditions tested, peas showed the greatest tendency to seed decay, followed by flax and then wheat. Flax and wheat were most subject to decay under low soil moisture, while peas were most subject to decay under conditions of high soil moisture.

EFFECT OF DIFFERENT SOIL TEMPERATURE LEVELS ON EMERGENCE

The temperature tests were carried out in temperature control tanks in the greenhouse, in which the soil temperature was maintained at 15°, 20°, 25° and 30°C and the air temperature at 15°C. Each seed type was tested separately, using 4 replicates and 25 seeds per crock. That is, there were 4 crocks with untreated seed and 4 crocks with seed treated with Ceresan M to act as checks, at each temperature level. The soil moisture was maintained at an optimum level for plant growth. The results are presented in Table II.

TABLE II

EFFECT OF DIFFERENT SOIL TEMPERATURE LEVELS ON THE EMERGENCE OF WHEAT, FLAX AND PEAS

Treatment	Number of Seedlings Emerged		
	Wheat	Flax	Peas
15°C	21.5	13.3xx	9.0xx
15°C Ceresan M	22.8	21.3	16.8
20°C	22.3	19.3	13.5xx
20°C Ceresan M	24.0	19.8	19.8
25°C	20.5	19.5	13.3xx
25°C Ceresan M	23.8	21.5	20.0
30°C	22.0	18.3xx	15.0xx
30°C Ceresan M	23.3	22.8	22.5

x significant between treated and untreated
xx very significant between treated and untreated

The emergence of wheat was not significantly reduced over that of the controls at any of the temperatures from 15° to 30° C. Flax emergence was very significantly reduced at 15° and 30° C. The lowest emergence in the pea test occurred at 15°C and the highest at 30°C, while the emergence at 20° and 25°C was intermediate and nearly equal. The emergence of peas was very significantly reduced over their controls at all the temperatures used.

Discussion

Wilson (44) carried out germination tests on blocks

of plaster of Paris, and found that 15°C was the optimum germination temperature of wheat. Higher temperatures of 20° and 30°C gave more rapid but not so complete germination. He found that mold attack on germinating wheat seed was of little importance at 15°C, but at 30°C the endosperm of the kernel is so affected that there is outward leaching of soluble food materials which are readily utilized by ever-present fungi. Fungal growth was favored at 30°C. He noticed an inverse relationship between the total percentage of germination and the percentage of moldy kernels.

Leach (21) states that the relative growth rates of the host and pathogen determine to a considerable degree the severity of preemergence infection at different temperatures. This growth rate effect would largely explain the fairly uniform emergence throughout the different temperature levels in the wheat experiment.

If the emergence at each temperature is totalled in the flax experiment, there is a slight increase in emergence with increasing temperature, with the lowest emergence at 15°C and the highest at 30°C. Thus, under these conditions 15°C appears to be the most unfavorable temperature at which to sow flax. There was considerable post-emergence blighting at 30°C so the most favorable temperature appears to be between 20° and 25°C.

The most unfavorable soil temperature for pea

emergence also appears to be 15°C, although the pea-decaying organisms seemed extremely active at all the temperatures used. They were likely more active at the higher temperatures, since for example, the plants at 30°C took five days to emerge while those at 15°C took nine days to emerge. If the time to emerge had been the same at all the temperatures, then it would be reasonable to assume that the greatest decrease in emergence would have occurred at the two higher temperatures. The effectiveness of chemical treatment seemed to increase with increasing temperature. Leach (21) found that seed decay and preemergence infection of garden peas were most severe between 12° and 25°C.

Wallace (40), Baylis (4) and Hull (15) concluded that soil temperature was less important than soil moisture in its effect upon pea seed decay and preemergence blight. In general, preemergence blight is most severe at temperatures that are relatively less favorable to the host than to the pathogen.

<u>Summary</u>

Under all soil temperatures used, peas showed the greatest tendency to decay, followed by flax and then wheat. The emergence of wheat was not significantly reduced at any of the temperatures from 15° to 30°C. Its optimum temperature would likely be about 20°C. The lowest emergence in flax and peas was at 15°C and the highest emergence was at 30°C.

EFFECT OF DATE OF SEEDING ON EMERGENCE

This experiment was carried out in the field using single row plots consisting of 12 foot rows, 1 foot apart; 100 seeds were sown in each row at a depth of 1½ inches. Each treatment was replicated four times in randomized blocks. Soil moisture analysis was carried out every other day and the soil temperature was taken every noon at seed level from the time of sowing until the emergence was recorded. Daily overnight low temperatures were also recorded. Soil moisture and temperature levels are listed in Table III.

TABLE III

SOIL MOISTURE AND TEMPERATURE LEVELS

	Early Seeding	Late Seeding
Average Soil Moisture	17.2%	20.3%
Average Noon Soil Temperature	20.6°C	20.5°C
*Average Overnight Low Soil Temperature	7.8°C	10.2°C

* The average overnight low soil temperature was taken at a depth of 4 inches, about 50 yards from the test plots.

The soil was rather dry for the first four days after seeding the first test, whereas the soil moisture was optimum throughout the second test. In general, the seedlings in the early test were exposed to a slightly lower soil temperature and to drier conditions than those in the late

seeding test. The results are presented in Table IV.

TABLE IV

EFFECT OF DATE OF SEEDING ON THE EMERGENCE OF WHEAT, FLAX AND PEAS IN THE FIELD

Treatment	Number of Seedlings Emerged	
	Early Seeding	Late Seeding
Wheat	85.0$_{x}$	75.5$_{x}$
Wheat Ceresan M ½ oz.	91.8	86.5
Flax	66.8$_{xx}$	67.5$_{x}$
Flax Ceresan M 1 oz.	80.0	76.5
Peas	27.8$_{xx}$	61.5$_{x}$
Peas Ceresan M 1 oz.	65.0	70.0

x significant between treated and untreated
xx very significant between treated and untreated

Early seeding improved the emergence of wheat, little affected the emergence of flax, but was detrimental to the emergence of peas. In all cases Ceresan M showed the greatest improvement in emergence in the early seeding. As in the previous experiments, wheat showed the least tendency to seed decay, followed by flax and then peas.

Discussion

The results of this date of seeding experiment illustrate well the value of seed treatment especially when the seeds are sown under conditions unfavorable for their

growth. Evidence of this is shown in the early seeding of peas and the late seeding of wheat. These results substantiate those of the previous moisture and temperature greenhouse experiments in that wheat proved the most tolerant of the various environmental conditions and peas the least tolerant.

The particularly low emergence from the untreated pea seed in the early seeding test could possibly be accounted for by the greater activity of Pythium species at the lower soil temperature. Ledingham (23) at Saskatoon obtained an abrupt decrease in emergence between the first and second pea sowing dates, while there was very little change in soil temperature. This worker suggested that P. ultimum and other organisms concerned in preemergence blighting of peas required a period in the spring to initiate active vegetative growth and that the early sown peas may have passed their susceptible stage before the fungi had increased sufficiently to cause much seed decay. McLaughlin (32) found that the percentage of Pythium isolates from the soil were generally high in the winter, spring and fall seasons and low in the summer. He showed that a combination of high soil temperature with low soil moisture generally resulted in a reduction in the percentage of Pythium isolates. If the first seeding had been two weeks earlier the results presented here may have been quite different.

The cultural study of peas is not a simple problem,

but the findings are mainly in agreement that seed protectants are beneficial, their value depending on seed and environmental conditions. In England and the United States of America, the cardinal factor in preemergence blighting has been shown to be excessive soil moisture and, perhaps more particularly, rain soon after planting in its tendency to compact the soil around the seed, providing moist, poorly aerated conditions. Soil temperature has been given a minor role by the English workers but in general they consider higher temperatures as being conducive to maximum emergence in garden peas. In the United States, McNew (33) found progressively less seed piece decay with each increase in temperature from 15° through 20° and 25° to 32°C. Ledingham (23), as well as Hutton (17) of Australia, found a tendency toward poorer emergence as the season advanced from cool spring to hot summer. Ledingham was able to correlate emergence with temperature but not with moisture.

<u>Summary</u>

Early seeding increased the emergence of wheat, little effected the emergence of flax and reduced the emergence of peas. Regardless of the time of seeding, wheat showed the least tendency to decay, followed by flax and then peas.

SUMMARY OF THE EFFECT OF ENVIRONMENTAL CONDITIONS ON EMERGENCE

Under all environmental conditions tested, peas showed the greatest tendency to decay and wheat the least, while flax was intermediate.

Wheat showed remarkable tolerance to its environment and especially to soil temperatures during its germination and emergence period. High emergence was discouraged by suboptimum soil moisture and was encouraged by optimum soil moisture, a temperature of about 20°C and early spring field sowing.

Low soil temperature and moisture favored preemergence blight of flax, while higher soil temperatures and moisture levels increased emergence. Field flax may be sown early in the spring if a seed protectant is used.

Peas, the most sensitive of these three grains to environmental conditions, readily succumb to high soil moisture (especially for the first three days after sowing) and to low soil temperatures. Good pea emergence was favored by low soil moisture and high soil temperature. Since the soil moisture-temperature balance appears to be a very delicate one, seed protectants should always be used on garden peas.

ISOLATIONS FROM ROTTED SEEDS

INTRODUCTION

In the previous section the influence of soil environmental conditions on the decay of wheat, flax and pea seed by microorganisms of the common soil microflora was considered. Isolation of some of these microorganisms from partially rotted seeds was attempted. This is discussed in the present section.

Many workers have isolated organisms carried by the seed. Out of 6,204 surface-sterilized samples of cereal seed, Machacek (28) recovered 43 genera and 102 species of fungi, the major portion appearing to be innocuous inhabitants of the seed. *Alternaria* was the most common genus and *Helminthosporium* the most common pathogen isolated. Greaney (13) associated wheat seed infected with *H. sativum* with low germination. Fungal hyphae may always be found between the epidermis and the inner pericarp of normal wheat seed (18, 37). Large numbers of certain species of epiphytic bacteria are commonly found on wheat (20, 24, 41).

Padwick (38) made isolations from surface-sterilized cotyledons of several varieties of peas grown in sterilized and unsterilized soil. He obtained an abundance of common molds and several pathogenic fungi, namely: a *Fusarium*

of the section Roseum, Fusarium culmorum and Botrytis cinerea. He concluded that the rotting of pea seeds in soil may be due to various fungi, and in nature it presumably would frequently be due to the combined effects of several, although the greater pathogenicity of the above mentioned pathogenic fungi in conjunction with their frequency of isolation and well-known wide distribution in soil would suggest their importance. Baylis (3) concluded, that species of Pythium were more important than those of Fusarium in causing pea seed decay.

METHODS

(a) Obtaining Partially Rotted Seeds

Non-treated seeds* of Red Bobs wheat, Redwing flax and Homesteader peas were sown in Edmonton black soil at a depth of 1 inch. The soil temperature was maintained in controlled temperature tanks at 15°, 20°, 25° and 30°C for wheat and at 20°C for flax and peas. The moisture content of the soil was kept at optimum or slightly above for plant growth. Seeds showing signs of decay or delayed germination were removed from the soil after the following intervals of time: 4 days from the 30°C tank, 7 days from the 25°C tank, 14 days from all the tanks and 28 days from the 15°C tank.

* The wheat and flax seed was harvested in 1949 and 1948, respectively.

(b) Isolation of Microorganisms From the Partially Rotted Seeds

Upon removal from the soil the seeds were surface-sterilized by submerging them for 1 minute in 70 percent ethyl alcohol and for 2 minutes in 1:1000 mercuric chloride, after which they were washed three times for a period of 5 minutes in sterile distilled water.

By means of sterile forceps the surface-sterilized seeds were placed in Petri plates containing potato dextrose agar at the rate of four seeds per plate. The plating procedure was varied in the following ways: by placing the seeds on hardened acidified medium, by completely submerging the seeds in acidified medium before it hardened, by placing the seeds on a hardened medium containing crystal violet, by completely submerging the seeds in a medium containing crystal violet before it hardened, and by placing the seeds on just-hardened medium. The acidification of the medium to pH 4 with 25 percent lactic acid inhibits the growth of bacteria, while a medium containing 1:125,000 crystal violet commonly inhibits fungal growth and has a bacteriostatic effect upon gram-positive bacteria.

The plated seeds were incubated at the same temperature as they had been kept at in the soil. Organisms growing from the seeds were transferred to potato dextrose agar slants and a record was kept of them.

(c) Testing the Isolates

A preliminary method using one or two replicates, was employed to test the seed-rotting capacity of these isolates. Surface-sterilized seed was inoculated with them, then sown in a sterile 3:1 Edmonton soil-sand mixture in $5\frac{1}{2}$ inch pots, at a depth of $1\frac{1}{4}$ to $1\frac{1}{2}$ inches, and at the rate of 25 seeds per pot. The pots for a given organism were held at the temperature at which that organism had been isolated. Organisms showing seed-rotting ability were retested using four replicates and a randomized block design.

(d) Seed Inoculations

The following methods of seed inoculation were employed in all the seed inoculation tests undertaken in the present investigation. The seed was always surface-sterilized, as previously described, before being inoculated.

For bacterial seed inoculation a 10 ml. water suspension of a 24 hour culture was poured on the seed. The seed was left to soak at room temperature for 2 hours, partially vacuumed for 15 minutes, then left to soak again for at least 2 more hours before sowing. The liquid inoculum was decanted off the seed on the sterile soil at seed level before the seeds were sown. This method is similar to that employed by Wallin (42) when working with *Xanthomonas translucens* var. *cerealis*, only he applied the vacuum pump immediately after the inoculum was added to the seed.

The fungal isolates were grown in 50 gm. of a sterile 10 percent cornmeal-soil medium in 250 ml. Erlenmeyer flasks at room temperature for a period of two to three weeks. The inoculum was then cut up on the flasks by means of a sterile spatula, poured out on sterile soil in pots and mixed with the top 3/4 inches of this soil. The seeds were sown on this mixture and then covered by more sterile soil. The contents of one Erlenmeyer flask were used to inoculate the seeds sown in one pot. The medium in the flasks and the soil in the pots were steam-sterilized at about 15 pounds pressure for three and eight hours, respectively. A 10 percent cornmeal medium had previously been used by Henry (14).

RESULTS*

A summary of the number of seed-decay organisms isolated from non-surface-sterilized wheat, flax and pea seed after varying periods of time in the soil at different temperatures is presented in Table V.

* The seed decay organisms, numerically designated, are listed in Appendix I.

TABLE V

NUMBER OF SEED-DECAY MICROORGANISMS ISOLATED FROM NON-SURFACE-STERILIZED WHEAT, FLAX AND PEA SEED* AFTER VARYING PERIODS OF TIME IN THE SOIL AT DIFFERENT TEMPERATURES

Type of Seed Used	Type of Organism Isolated	Temperature of Soil in °C in Which Seeds Were Rotted	Number of Days Seeds Were in the Soil	Number of Organisms Isolated	Number of Organisms Able to Significantly Decrease Emergence
Wheat	Bacteria	15°	14	16	0
		15°	28	15	0
		20°	14	32	1
		25°	7	1	0
		25°	14	2	0
		30°	4	2	0
		30°	14	1	0
Wheat	Fungi	15°	14	39	9
		15°	28	28	1
		20°	14	79	10
		25°	7	23	11
		25°	14	29	4
		30°	4	23	0
		30°	14	29	2
Flax	Bacteria	20°	14	13	0
Flax	Fungi	20°	14	42	6
Pea	Bacteria	20°	14	24	0
Pea	Fungi	20°	14	23	1

* The seed was not surface-sterilized before it was sown.

According to these results, more fungi than bacteria were isolated from the rotted seeds and more of the former than the latter were able to reduce emergence significantly. Not only were more organisms isolated at the lower soil temperatures, but more of the organisms isolated at these temperatures appeared to possess seed-rotting ability; this could, of course, be due to the slower growth rate of the host plants at the lower soil temperatures.

There doesn't appear to be any correlation between the number of days wheat seed was in the soil and the number of bacteria isolated from the seeds. Seed kept at a soil temperature of 20°C for a period of fourteen days produced the greatest number of bacterial isolates and yielded the only bacterial isolate that was able to reduce the emergence of wheat significantly.

In general, at all the soil temperatures used, the number of fungal saprophytes isolated from wheat seed increased directly with the period of time the seed was in the soil. Fungi capable of causing severe preemergence blighting of wheat were isolated from rotting seed at all the soil temperatures tested, although the number isolated from soil at 30°C was rather low.

The number of fungi and bacteria isolated from rotting pea seed was nearly equal. There were over three times as many fungi as bacteria isolated from rotting flax

seed. Although peas proved the most susceptible to seed decay, a very low portion of the fungi isolated from them were capable of rotting their seeds. None of the bacteria isolated from flax or pea seed rotted these seeds under the conditions tested.

The above discussion is based solely on the results obtained and should be evaluated as such. It is realized that any variation in materials or methods, such as in chemicals used for surface sterilizing the seeds, or media employed, would possibly have a marked effect on the results obtained.

SUMMARY

The fungi surpass the bacteria in the number isolated from the rotted seeds and in the number able to reduce significantly the emergence of wheat, flax and peas. The majority of the seed-decay organisms were isolated at the lower soil temperatures. The number of saprophytic fungi isolated from wheat seed increased directly with the period of time the seed was in the soil. Wheat seed-decay fungi were isolated at all the soil temperatures tested. A very low percentage of the fungi isolated from decaying pea seed were able to reduce the emergence of peas significantly.

SEED ROTTING CAPACITY OF FUNGAL ISOLATES UNDER VARIOUS CONDITIONS

INTRODUCTION

In the previous section fungi capable of significantly reducing the emergence of their host when applied to seed were isolated from rotting wheat, flax and pea seed. The purpose of this section is to discuss the ability of these fungi to rot seeds of the crop plants in question under different conditions.

METHODS

The experimental methods used in this series of tests were the same as those described in the previous section.

EFFECT OF SOIL TEMPERATURE

The fungi that were isolated from decaying wheat seed at the various soil temperatures and found pathogenic, were tested for seed-rotting ability at different soil temperatures. Temperature may alter the susceptibility of the host or the pathogenicity of the microorganism. Dickson (9) found that the seedling blight organism, *Gibberella saubinetii*, would only produce severe infection of corn at low temperatures and of wheat at high temperatures. In this case

temperature influenced the susceptibility of the host by altering it chemically. Low temperatures are known to increase the disease-producing capacity of several fungi including Rhizoctonia solani, Pythium ultimum and Tilletia caries, while higher temperatures increase the pathogenicity of Helminthosporium sativum and Cladosporium fulvum. Leach (21) found preemergence infection most severe at temperatures that were relatively less favorable to the host than to the pathogen as measured by the ratio of their growth rates.

A comparison was made of the ability of the fungi that were isolated in the present studies from wheat seed held at soil temperatures of 15°, 20° and 30°C, to reduce the emergence of wheat at these soil temperatures.

After the emergence data was analyzed by the analysis of variance, the mean of each treatment was converted to a percentage preemergence blighting on the basis of its check. The following formula was used:

$$\frac{x - y}{x} \times 100 = \text{percent blighting}$$

where x = the percent of emergence in the check

y = the percent of emergence in the treatment.

This method was introduced by Abbott (1) and it seems to offer a reliable means for comparing results when several series of experiments have been carried on, each based on a different check. A summary of the results is presented in Table VI.

TABLE VI

ABILITY OF FUNGI ISOLATED FROM ROTTING WHEAT SEED AT SOIL TEMPERATURES OF 15°, 20° and 30°C, TO PRODUCE PREEMERGENCE BLIGHT OF WHEAT AT THESE SOIL TEMPERATURES

Soil Temperature at Which the Organisms Were Isolated	Organism	Percentage of Preemergence Blighting in Relation to Uninoculated Checks		
		Soil Temperature		
		15°C	20°C*	30°C
15°C	135	34.4xx	36.5xx	55.6xx
	136	40.4xx	36.5xx	62.2xx
	137	17.4x	32.6xx	67.8xx
	138	17.4x	24.2x	53.9xx
	139	19.7x	33.7xx	37.2xx
	310	30.9x	0.0	0.0
	332	86.2xx	0.0	11.1
	345	75.7xx	11.2	30.6xx
20°C	114	39.5xx	42.3xx	22.5
	127	30.2xx	42.3xx	22.5
	143	35.8xx	34.2xx	16.7
	145	40.5xx	15.8x	41.7
	146	38.1xx	24.7xx	16.7
	149	30.2xx	19.5xx	10.0
	264	31.2xx	28.9xx	47.5
	268	38.1xx	30.0xx	14.2
	281	28.8xx	16.8x	26.7
	286	42.8xx	23.7xx	14.2
30°C	159	12.3	7.1	24.5
	172	13.8	4.4	24.5
	240	12.3	0.0	60.9xx
	247	17.2	11.5	29.1x

x significant
xx very significant

* The 20°C soil temperature level in the experiments where the organisms isolated at 15° and 30° were employed was not constant.

The majority of the fungi that were isolated at a soil temperature of 15°C were quite active at all soil temperatures tested. In general, the ability of the isolates 135 to 139, inclusive, to produce preemergence blight of wheat increased with increasing soil temperature, while the 310 and 332 isolates appeared relatively ineffective at the two higher temperatures. The 345 isolate significantly reduced the emergence only at 15° and 30°C, being over twice as effective at the lower temperature. The following isolates, in decreasing order, caused the most preemergence killing: 332, at a soil temperature of 15°C, 137, at a soil temperature of 30°C, and 135 and 136, at a soil temperature of 20°C. Six out of these eight isolates showed a wide adaptability to the various soil temperatures.

All the isolates that were obtained at a soil temperature of 20°C significantly reduced the emergence of wheat at a soil temperature of 15°C but not at a soil temperature of 30°C. Only isolates 114 and 127 of these ten isolates were more effective in causing preemergence blighting at 20°C than at a soil temperature of 15°C. There might have been significant reductions in emergence at a soil temperature of 30°C if there had been greater uniformity within the treatments and if the emergence of the checks had not been so low. On a percentage basis of the means, isolates 145 and 264 caused more preemergence blighting at a soil temperature of 30°C than at the other soil temperatures.

The organisms isolated at a soil temperature of 30°C were incapable of causing significant reductions in emergence at soil temperatures of 15° and 20°C. The emergence in all the treatments at 20°C was low and nearly equal.

In comparing the organisms isolated at the various soil temperatures, those isolated at 15°C contain the outstanding ones so far as seed-rotting ability is concerned. While isolates 332 and 345 don't appear to possess so wide a tolerance to the various soil temperatures as some of the other organisms, they were capable of causing the greatest decrease in emergence. Isolates 135 and 136 possess a high degree of pathogenicity and a wide tolerance to the various soil temperatures, since they caused the greatest average decrease in emergence, taking into consideration all of the temperatures tested. The majority of the organisms isolated at 15°C were more adaptable to the different soil temperatures than those isolated at 20°C, while the 30°C isolates appeared to be active only at or near that temperature.

Discussion

These preemergence-blighting fungi of wheat, appear to have a sufficiently wide temperature range to enable them to operate quite effectively at the soil temperatures in which wheat is generally sown. Sowing wheat when the soil temperature was at a particular level would therefore not be a very satisfactory means of avoiding damage completely, but

it might be used to reduce damage.

Summary

Five out of the eight pathogenic organisms that were isolated from rotting wheat seed at a soil temperature of 15°C significantly reduced the emergence of wheat at soil temperatures of 15°, 20° and 30°C. The pathogenicity of these five organisms increased with increasing soil temperature. The organisms isolated at 20°C proved most pathogenic at 15°C and relatively non-pathogenic at a soil temperature of 30°C. The seed-rotting ability of the fungi isolated at a soil temperature of 30°C was quite specific for that temperature. In general the organisms isolated at a soil temperature of 15°C were least specific in their reactions to soil temperature and those isolated at 30°C were most specific to soil temperature while the 20°C isolates were intermediate.

EFFECT OF SOIL MOISTURE

Although all fungi are classified as being aerobic, some are known to thrive under near-anaerobic conditions. It is the purpose of this experiment to find the level of soil moisture most conducive to preemergence blighting.

Machacek (26), while experimenting with wheat seedlings in non-sterile soil, found that if the soil was kept too moist the seedlings were frequently attacked by

species of Pythium and damped off, and if the soil was kept too dry, the seedlings were occasionally attacked by Rhizoctonia solani. His results show that with a decrease in the amount of water given a seed bed, there was an increase in the amount of blighted seedlings. Although Gibberella saubinetii and some Fusaria are favored by low soil moisture, other Fusaria, Helminthosporium sativum and Pythium species are favored by high soil moisture (11).

The test organisms used in this experiment were fungi that had been isolated from rotting wheat seed at a soil temperature of 20°C. Red Bobs wheat was sown at the rate of 25 seeds per pot. The soil moisture was maintained at three different levels. Soil moisture analyses were made every other day and water was added as necessary to maintain the desired level. The mean moisture levels were 14.5%, 21.3% and 26.3% for low, optimum and high, respectively. The air temperature of the greenhouse varied from a daily mean high of 26.5°C to a daily mean low of 11.9°C. The three moisture tests were run concurrently on one bench in the greenhouse. Significant differences in the emergence were obtained by means of a split-plot analysis. The results are presented in Table VII.

TABLE VII

COMPARISON OF THE ABILITY OF THE FUNGI ISOLATED FROM ROTTING WHEAT SEED AT A SOIL TEMPERATURE OF 20°C TO CAUSE PREEMERGENCE BLIGHT OF WHEAT UNDER LOW, OPTIMUM AND HIGH SOIL MOISTURE CONDITIONS

	Average Number of Seedlings Emerged*		
Organism	Low Soil Moisture	Optimum Soil Moisture	High Soil Moisture
114	12.8	11.3	13.0
127	18.5	11.5	13.5
143	12.3	13.5	10.8
145	13.3	12.3	14.0
146	14.0	15.5	11.5
149	12.8	12.8	11.8
264	14.5	15.0	12.0
268	14.8	11.5	13.5
281	16.3	13.3	11.8
286	14.3	14.3	11.3
Check	14.5	16.8	9.3

* Based on 4 replicates, 25 seeds per replicate.

There was a highly significant difference in the emergence of the wheat seedlings at the three moisture levels. The greatest preemergence blighting occurred under high soil moisture conditions and the lowest under low soil moisture conditions, while the preemergence blighting under optimum soil moisture conditions was intermediate. There were no significant reductions in emergence by any of the organisms, due possibly to the low emergence in the checks. The low emergence in the checks was probably due to early contamination after seeding by a multitude of microorganisms which flourished in the sterile soil and cornmeal-soil medium at the relatively high greenhouse temperatures.

Discussion

These results are quite opposite to those obtained by Machacek (26), and to the results of an earlier experiment reported in this paper, when the greatest decrease in the emergence of wheat was obtained under low soil moisture conditions. It should be noted though, that the above contrary results were obtained when using non-sterile soil. It is not reasonable to expect the same results in non-sterile soil with its compliment of complex microflora, as in sterilized soil containing a single parasitic fungus.

Summary

There was a highly significant difference in the emergence of the wheat seedlings at the three moisture levels.

The lowest emergence occurred under high soil moisture conditions and the highest under low soil moisture conditions, while the emergence under optimum soil moisture conditions was slightly higher than intermediate. No other significant differences were observed.

REACTION OF DIFFERENT TYPES OF SEED TO THE SAME FUNGI

We have seen how these isolates from rotting seed react to different soil temperature and moisture levels. A major aim of this study was to ascertain their relative reactions on different types of seed. That is considered in this section. Would these fungal isolates, for instance, have the necessary enzyme compliments to enable them to attack a seed that was high in starch like wheat as aggressively as ones that were high in lipoids like flax, or high in proteins like peas? In an attempt to answer this question, preemergence-blighting fungi isolated from each type of rotting seed at a soil temperature of 20°C were tested against the two other types of seed. Padwick (38) found several fungi pathogenic to pea cotyledons that were not isolated from peas. Greaney (13) isolated *Helminthosporium sativum* from wheat, barley and rye seed, and found that it was only associated with low germination in naturally infested wheat seed. The same seed and methods were used in this experiment as were employed in the previous experiments. Tests involving the isolates from flax and pea seed were

carried out concurrently against the three types of seed as two separate experiments, whereas the isolates from wheat seed were tested against the three types of seed in individual experiments. In order to make an overall comparison of the emergence it was therefore necessary to convert the number of seedlings emerged to the percentage of preemergence blighting on the basis of their individual checks. This method was previously described on page 26. The results are presented in Table VIII.

TABLE VIII

COMPARISON OF THE PREEMERGENCE BLIGHTING OF WHEAT, FLAX AND PEA SEED BY FUNGI ISOLATED FROM DECAYING WHEAT, FLAX AND PEA SEED AT A SOIL TEMPERATURE OF 20°C

Seed Type From Which Organisms Were Isolated	Organisms	Percentage of Preemergence Blighting in Relation to Uninoculated Checks		
		Wheat	Flax	Peas
Wheat	114	42.3xx	30.8x	31.9xx
	127	42.3xx	9.2	12.9
	143	34.2xx	81.1xx	42.9xx
	145	15.8x	74.1xx	20.0
	146	24.7xx	45.9xx	20.0
	149	19.5xx	57.8xx	2.4
	264	28.9xx	63.2xx	21.4
	268	30.0xx	32.4x	7.1
	281	16.8x	22.7	0.0
	286	23.7xx	16.2	16.7
Flax	F120	9.9	93.5xx	0.0
	F124	7.4	87.8xx	0.0
	$F213_R$	54.2xx	97.6xx	5.9
	$F213_G$	18.7x	85.4xx	12.9
	$F236_R$	50.7xx	44.7xx	1.2
	$F236_G$	11.3	83.7xx	38.2xx
Pea	P268	0.0	21.7x	10.0
	P270	4.0	90.9xx	36.0xx

x significant
xx very significant

The wheat preemergence blighting organisms were able to rot the seeds of flax much more easily than the seeds of peas. In fact, the isolates from 143 to 264, inclusive, which comprise 50 percent of the wheat isolates tested, caused more severe preemergence killing of flax than of wheat. Only isolates 114 and 143 were able to significantly reduce the emergence of peas and these were the only isolates from wheat that were capable of significantly reducing the emergence of the three types of seed. Organisms 127, 281 and 286 appear to be specific wheat seed decay organisms in that they were unable to significantly reduce the emergence of flax or peas.

Out of the six preemergence-blighting organisms isolated from flax seed, only three: $F213_R$, $F213_G$ and $F236_R$ were capable of significantly reducing the emergence of wheat and only one, $F236_G$, was able to significantly reduce the emergence of peas. Isolates F120 and F124 appeared specific for flax. None of the flax isolates were able to significantly reduce the emergence of the three types of seed.

Neither of the isolates obtained from pea seed were capable of preemergence killing of wheat, only one significantly reduced the emergence of peas, while they both significantly reduced the emergence of flax.

Discussion

From these results it would appear that many similar

fungi are capable of causing significant preemergence killing of wheat and flax, and that only a few of these fungi are able to cause a significant reduction in the emergence of peas. This seems a little irregular, since earlier tests showed peas the most susceptible of these three seed types to seed decay. Padwick (38) looked upon the rotting of pea seed in the soil as a complex disease in which more than one distinctly parasitic fungus may be playing a part. He was able to show, however, that the following fungi from wheat were highly pathogenic to pea cotyledons: Fusarium avenaceum, F. culmorum, F. graminearum and Helminthosporium sativum.

Of the three types of seed exposed, flax appears to be the most susceptible to preemergence blighting by the various fungal isolates. It is interesting to note that a fungus which was isolated from decaying pea seed and was relatively unable to rot that seed, caused significant preemergence blighting of flax.

It a rotation plan to minimize preemergence blighting was based on these results, it would not seem advisable to sow flax in infested soil after wheat or peas. It would also not be advisable to rotate wheat with flax, but peas would be fairly safe on infested soil upon which flax or wheat had grown.

Summary

Out of the ten organisms which were isolated from

decaying wheat seed and found to be able to significantly reduce the emergence of wheat, seven were able to reduce the emergence of flax and only two were able to reduce the emergence of peas. Three organisms were found specific to wheat, while two were able to cause significant preemergence blighting in wheat, flax and peas. Of the six preemergence-blighting organisms isolated from flax, three were found to significantly reduce the emergence of wheat and only one the emergence of peas. Two were specific to flax and none were able to significantly reduce the emergence of the three types of seed. Neither of the isolates obtained from pea seed were able to cause preemergence killing of wheat, only one significantly reduced the emergence of peas, while both significantly reduced the emergence of flax. Flax appeared to be the most susceptible to the various isolates. Preemergence blighting of flax and wheat may be caused by a fairly similar group of fungi. Only two out of the eighteen isolates tested were capable of causing a significant reduction in the emergence of the three types of seed, ten caused seed decay of two types of seed and five were specific to the type of seed from which each was isolated.

EFFECT OF MISCELLANEOUS FACTORS ON SEED DECAY

All the previous tests with the isolates were carried out using sterile soil and normal seed. The following experiments were designed to determine how one of the pre-

emergence-blighting fungi would act in non-sterile soil, on seed treated with a mercurial dust, on injured seed, and on normal seed after it had been sown in a soil medium containing ground seed of the same type.

Machacek (26) found that the amount of soil-borne infection of cereal seedlings by soil-borne organisms was negligible when friable, non-sterile soil was kept moist and at 20°C. Steam sterilizing changes the physical condition of the soil and if it is prolonged, toxic amounts of ammonia may be released.

Hurd (16) found that if wheat is injured over the endosperm, 100 percent fatal infection results when the spores of Penicillium or Rhizopus are present; but if the injury is over the embryo, the seeds remain practically immune. Field and greenhouse tests made at the University of Alberta, using non-inoculated seed in non-sterile soil, have shown that the reduction in emergence is greater when the injury is over the embryo than over the endosperm. Previous work at this laboratory has also shown that an increase in emergence due to seed treatment with a mercurial fungicide, is greatest with embryo-injured seed, least with normal seed and intermediate with endosperm-injured seed.

The medium upon which the parasite has grown may have quite an effect upon it. In the laboratory a fungus may be forced to live on artificial substrates for long

periods. Its diet is often unbalanced there since it is frequently overfed with carbohydrate and nitrogen compounds. In the sudden transference onto a formerly suitable host plant, the conversion to the original parasitic mode of life cannot always be accomplished rapidly enough. Adaptive enzymes, which some fungi possess, must therefore be brought into play. Fomes igniarius, the false tinder fungus of apple trees, is a more successful parasite when it has previously been grown on wood from suitable trees than if it had been cultured on bread or agar. Gibberella saubinetii, after previous culture on oatmeal, killed only 10 percent of Pinus seedlings, whereas after culture on steamed rice under the same external conditions, it killed 50 percent of the seedlings (12).

Only wheat was used in this experiment, the endosperm of which was injured by cutting a niche with a scalpel approximately ½ mm. wide, 1½ mm. long and ½ mm. in depth, midway on the side of the kernel. Injury to the embryo was accomplished by severing the testa over it longitudinally with a dissecting needle. All the other seeds used in this experiment were hand-picked to ensure their soundness. Ceresan M, an organic mercurial fungicide, was used to treat the seed. It was applied at the rate of ½ oz. per bushel. The wheatmeal-soil medium contained 10 percent ground wheat seed. The test organism chosen was one that had been isolated from rotting wheat seed at a soil temperature of 20°C.

During the course of this experiment the greenhouse temperature varied from a daily high mean of 26.1°C to a daily low mean of 11.8°C. A summary of the results is presented in Table IX.

TABLE IX

ABILITY OF A FUNGUS ISOLATED FROM A ROTTING WHEAT SEED AT A SOIL TEMPERATURE OF 20°C TO PRODUCE PREEMERGENCE BLIGHT OF WHEAT UNDER DIFFERENT SOIL, SEED AND MEDIA CONDITIONS

Treatment	Average Number of Seedlings Emerged*
Isolate 143 Against Normal Seed in Sterilized Soil	14.0
Check Normal Seed in Sterilized Soil	14.5
Isolate 143 Against Normal Seed in Non-Sterilized Soil	15.0
Check Normal Seed in Non-Sterilized Soil	13.0
Isolate 143 Against Ceresan M Treated Normal Seed in Non-Sterilized Soil	15.3
Check Ceresan M Treated Normal Seed in Non-Sterilized Soil	18.0
Isolate 143 Against Embryo-Injured Seed in Sterilized Soil	4.5
Check Embryo-Injured Seed in Sterilized Soil	6.5
Isolate 143 Against Endosperm-Injured Seed in Sterilized Soil	11.8
Check Endosperm-Injured Seed in Sterilized Soil	15.5 x
Isolate 143, Previously Grown on Wheatmeal-Soil Medium, Against Normal Seed in Sterilized Soil	14.3
Check Wheatmeal-Soil Medium and Normal Seed in Sterilized Soil	18.8 x

x significant
xx very significant
* Based on 4 replicates, 25 seeds per replicate

The low emergence in the sterile soil check plots was probably due to early post-seeding contamination of the sterile cornmeal-soil medium, since a similar decrease in emergence was obtained when this inoculum was placed in non-sterile soil. Further evidence of this is that a highly significant increase in emergence was obtained when Ceresan M treated seed was sown in non-sterile soil. Although these contaminating organisms were very effective in reducing emergence, the seed treatment appeared quite effective against them and relatively ineffective against isolate 143.

Whether isolate 143 was present or not, the emergence of endosperm-injured seed was very significantly better than that of embryo-injured seed, and nearly equal to that of normal seed. Isolate 143 significantly reduced the emergence of endosperm-injured seed over its check, but not that of embryo-injured seed.

After previous growth on a wheatmeal-soil medium, isolate 143 significantly reduced the emergence of wheat over its check. This could either mean an increase in its pathogenicity due to the medium or that the wheatmeal-soil medium proved less favorable to contaminants than did the cornmeal-soil medium.

Discussion

These results stress that one cannot be too cautious in avoiding contamination when using sterile soil

under relatively high temperature conditions. Similar methods proved satisfactory under moderate temperature conditions. The relative predisposition of seed to decay by mechanical injury, was found to be similar to that obtained by earlier experiments in this laboratory, and different from the results obtained by Hurd (16), in that the embryo-injured seed appeared to be much more liable to preemergence blighting than endosperm-injured seed. Since the Ceresan M seed treatment was ineffective in preventing preemergence blight by isolate 143, it would suggest that this isolate attacked the seedling rather than the seed itself. It is of practical significance that the normal soil microflora did not appear to nullify the preemergence blighting ability of isolate 143.

Summary

Although Ceresan M was non-effective against isolate 143, it significantly increased the emergence of normal wheat in non-sterilized soil. Regardless of treatment, embryo-injured seed showed the lowest emergence, while the emergence of endosperm-injured seed was nearly equal to that of normal seed. The fungus isolate 143 significantly reduced the emergence of endosperm-injured seed, and of normal seed after it was grown on a 10 percent wheatmeal-soil medium, over their checks. This fungus also significantly reduced the emergence of embryo-injured seed over endosperm-injured seed that had been inoculated with it.

SUMMARY OF THE SEED-ROTTING CAPACITY OF THE DIFFERENT FUNGAL ISOLATES UNDER VARIOUS CONDITIONS

Preemergence blighting fungi that were isolated from decaying wheat seed at soil temperatures of 15°, 20° and 30°C were tested for their ability to rot wheat seed over this range of temperatures. In general these isolates proved quite active at all the soil temperatures tested. Those isolated at 15°C were the most active and those isolated at 30°C the least active.

The wheat seed-decaying fungi that were isolated from rotting wheat seed at a soil temperature of 20°C were tested against wheat seed at low, optimum and high soil moisture levels. Highly significant differences in emergence were obtained between each of the moisture levels, with the lowest emergence in high soil moisture and the highest emergence in low soil moisture, while the emergence under optimum soil moisture conditions was intermediate.

The fungi that were isolated from decaying wheat, flax and pea seed at a soil temperature of 20°C were tested against each of these seeds. The majority of the fungi isolated from either flax or wheat seed were able to cause significant preemergence blighting of flax and wheat but not of peas. Of the eighteen isolates tested, the following were capable of causing a significant reduction in emergence: two from wheat of the three seed types, ten of two seed

types, and five were specific to the seed types from which they were isolated. Flax appeared to be the most susceptible to the various isolates and peas the least susceptible. The majority of these fungi were non-specific to one seed type.

When one 20°C wheat isolate was tested against wheat seed under various conditions, it was found to significantly reduce the emergence of endosperm-injured seed, and of normal seed after it was grown on a 10 percent wheatmeal-soil medium, over their checks. It also significantly reduced the emergence of embryo-injured seed over endosperm-injured seed. Regardless of treatment, embryo-injured seed showed the lowest emergence, while the emergence of endosperm-injured seed was nearly equal to that of normal seed. Although Ceresan M was non-effective against isolate 143, it increased the emergence of normal wheat to a highly significant degree in non-sterile soil.

PERSISTENCE OF CHEMICAL SEED PROTECTANTS ON SEED IN THE SOIL

INTRODUCTION

The foregoing work demonstrated the value, and strongly suggested the need, of seed protectants when certain plants are grown from seed in soil. Much work has been done on the testing of various substances as seed protectants (6, 8, 19, 31), on determining suitable rates of application and wider uses of substances which show promise as seed dressings (8, 30); also on their relative toxicity to pathogens (46, 35), plants (8), and animals. Work has also been done on their seed-adhering properties when used with inert dusts (10, 43), with water (10, 22), or with oil (10); on their decomposition in soil (5, 7), and on their effect on the soil flora (5); on the accuracy and uniformity of application by means of commercial seed treating machines (27), and on methods of assaying them (2, 22, 25, 27, 29, 34, 39). To the writer's knowledge, no work has been published on the persistence of these seed fungicides on seed in the soil. Therefore such studies were undertaken and are discussed in the present section.

METHODS

Wheat, flax and pea seeds were sown within three days after treatment with commercial seed protectants, at a depth of $1\frac{1}{2}$ inches in a 3:1 Edmonton soil-sand mixture, in 5 inch pots at the rate of at least 25 seeds per pot. The seed used was Red Bobs wheat, Redwing flax and Homesteader peas. The pots were placed on an open bench in the greenhouse and watered daily.

At daily intervals four seeds were removed from the soil of each seed treatment and freed of growth and adhering soil. These were placed in a Petri dish containing a suspension of potato dextrose agar and the test fungus, just before the agar hardened. The plates were incubated at about 25°C for 24 - 36 hours. The persistence of the fungicides was determined by inverting the Petri plates and measuring the average radius of the zones of inhibition of the test fungus around the seeds. The test fungus had been isolated from a decaying wheat seed at a soil temperature of 20°C. Although it proved non-pathogenic to wheat, it is a profuse producer of spores, grows rapidly and in general serves very well as a test organism.

About 8 ml. of agar was added to Petri plates used for wheat and flax, and about 10 ml. to those used for peas. The wheat and flax seeds were completely submerged in the agar, whereas the pea seeds were only partially embedded in

it. A large loopful of spores (and probably some mycelium) of the test organism was suspended in 10 ml. of sterile distilled water, and 1 ml. of the suspension was added to each Petri plate. Observations were made of the amount of growth from the seed and the condition of the seed when it was removed from the soil.

RELATIVE PERSISTENCE OF CERESAN M ON VIABLE AND NON-VIABLE WHEAT, FLAX AND PEA SEED IN SOIL UNDER GREENHOUSE CONDITIONS

This experiment was carried out to compare the relative persistence of Ceresan M on viable and non-viable wheat, flax and pea seed in the soil, and to check the possibility of antifungal substances being produced by germinating seed. If non-viable seed produced results similar to viable seed, it was planned to use the former in the subsequent tests.

The non-viable seeds were prepared by exposing viable seeds for two minutes to 15 pounds steam pressure in an autoclave, after which they were dried at room temperature. Ceresan M was applied at the rate of 1 oz. per bushel for flax and peas, and ½ oz. per bushel for wheat. The seeds were manually shaken for a period of five minutes in stoppered containers after the fungicide was added, to help ensure an even distribution. The stoppers were then removed. The other methods employed are similar to those previously des-

cribed on page 49. A summary of the results is presented in Table X.

TABLE X*

RELATIVE PERSISTENCE OF CERESAN M ON VIABLE AND NON-VIABLE WHEAT, FLAX AND PEA SEED IN SOIL UNDER GREENHOUSE CONDITIONS

Treatment	Radius of the Zone of Inhibition in mm. After 0 - 5 Days in the Soil					
	0	1	2	3	4	5
Wheat Viable Ceresan M ½ oz.	8.0	3.0	2.3	1.5	1.0	0.0
Wheat Non-viable Ceresan M ½ oz.	8.5	2.0	0.0			
Flax Viable Ceresan M 1 oz.	7.3	2.0	0.0			
Flax Non-viable Ceresan M 1 oz.	4.5	0.0	0.0			
Peas Viable Ceresan M 1 oz.	21.0	3.3	0.0			
Peas Non-viable Ceresan M 1 oz.	21.5	2.3	0.0			

* The results for the untreated viable and non-viable seed, which served as checks, were omitted from the above table since they caused no inhibition of the test organism.

After seed treatment the amount of Ceresan M adhering to the non-viable seed, as indicated by the size of the zone of inhibition, was much less on flax, and slightly more on wheat and peas than that on viable seed. After one day in the soil the amount of seed dressing persisting on non-viable seed was less than that on viable seed for all

the seed types. Viable wheat exhibited the greatest ability to retain the fungicide, since after four days in the soil it produced a trace of a halo, whereas all the other treatments failed to produce any inhibition after the seeds were two days in the soil.

The wheat seed treated with Ceresan M made slightly more rapid growth than the untreated seed. Seed treatment had little effect on the growth of flax and peas. By the fourth day in the soil, the viable flax seeds were discarding their seed coats. Much trouble was experienced from soil adhering to the non-viable, seed treated and non-seed treated flax and pea seed. By the fifth day in the soil, the non-viable, non-treated pea seeds were very water-soaked, fell apart easily, and the seed coat tended to be shed with the adhering soil. Ceresan M appeared relatively ineffective in preventing the decay of non-viable pea seed. Such seed proved more subject to decay than untreated, viable pea seed.

Discussion

Due to the variance in the ability of the fungicide to adhere to non-viable seed as compared to viable seed, and to its more rapid deterioration in the soil, it would appear inadvisable to use non-viable seed in any subsequent tests of this sort. Hurd (16) found that death renders previously immune wheat seeds immediately susceptible to attack by

Penicillium and Rhizopus. It does not appear to be an easy matter to compare the relative adhesiveness of the fungicide on the three types of seed by the method employed in this paper due to the difference in the size of their seed.

This type of bioassay, though simple, is not without faults. Some contamination occurred in the Petri plates from soil adhering to the seed and likely from the seed itself. The frequency of contamination increased with increasing time the seed was in the soil. Probably the easiest way to avoid this contamination would be to employ a test organism that would grow rapidly in a very acidic medium at low temperatures. In preliminary tests the size of the zone of inhibition was found to vary with the concentration of the test organism and its concentration would vary with the amount of agar added to the plate. The radius of the halos was also found to decrease in size, to the average extent of 1 mm., during the period of incubation from 24 to 48 hours. Thornberry (39), when using paper disks on the surface of the agar to test toxicants, found that with a relatively thick layer of "seeded" agar, the zones of inhibition would have a cone-shaped, poorly defined margin which was difficult to measure. Other factors affecting the size of the halo would be: uniformity of coverage of the fungicide (27, 43); the type of filler, its state of subdivision and whether or not substances of an adhesive nature had been added (10, 43); the interval between treating and seeding (27); also the

amount of agitation the treated seed was exposed to before seeding (10). Fitzgibbon (10) demonstrated that the general adhesive characteristics of seed dressings are fairly well maintained from one variety of seed to another, so that a dressing satisfactory for any one cereal tested will be satisfactory for all in this respect.

Summary

Ceresan M did not persist as well in the soil on non-viable wheat, flax and pea seed as it did on the same types of viable seed. Regardless of treatment the non-viable seed was more subject to decomposition in the soil than the viable seed. Of the seed types tested, viable wheat exhibited the greatest ability to retain the fungicide, since after four days in the soil it produced a trace of a halo, whereas all the other treatments failed to produce any inhibition of the test organism after two days in the soil. The untreated viable and non-viable seed failed to inhibit the growth of the test organism.

RELATIVE PERSISTENCE OF VARIOUS SEED PROTECTANTS ON VIABLE WHEAT, FLAX AND PEA SEED IN THE SOIL UNDER GREENHOUSE CONDITIONS

This experiment was designed to enable a comparison between commercial seed dressings which contain mercury and non-mercurial preparations, and between dry and wet methods

of treatment; as measured by the ability of these seed dressings to persist on viable wheat, flax and pea seed in the soil under greenhouse conditions.

Manufacturers are endeavoring to produce fungicides which possess high toxicity to microorganisms and low toxicity to higher animals. This quality is found to a greater extent in many of the non-mercurial than in the mercurial preparations. The hazard to humans of inhaling poisonous dusts while treating grain has been overcome by using some of the preparations in liquid form.

The methods employed in this experiment were similar to those described on page 49, and seed from the same stock was used. The trade names of the fungicides used, along with their active ingredients and rates of application, are listed in Table XI.

TABLE XI

TRADE NAMES, ACTIVE INGREDIENTS, AND RATES OF APPLICATION OF THE FUNGICIDES

Trade Name	Active Ingredients	Rates of Application**		
		Wheat	Flax	Peas
Ceresan M*	7.7% ethyl mercury P-toluene sulfonanilide 3.2% mercury equivalent	½ oz.	1 oz.	1 oz.
Panogen 14	methyl mercury dicyandiamide 1.4% mercury equivalent	0.04 ml./ 50 gm. seed	0.07 ml./ 50 gm. seed	0.07 ml./ 50 gm. seed
C.I.L. Bunt Cure	40.0% hexachlorobenzene	½ oz.	2 oz.	2 oz.
MTH	75.0% N-nitroso phthalimidine 25.0% pyrophyllite	1 oz.	2 oz.	2 oz.
Orthocide 406	50.0% N-trichloromethylthio-tetrahydrophthalamide	½ oz.	2 oz.	2 oz.

* The rates of application of Ceresan M used as a slurry were those recommended by Canadian Industries Limited; 0.1267 ml. and 0.5066 ml. of a 32.94% solution were added to 25 and 100 gm. lots of flax and peas, respectively, by means of a micropipette. The rate for wheat was 0.1267 ml. of a 10.25% solution to 25 gm. of seed.

** rates per bushel in ounces

The other rates of application are either those recommended by the manufacturer, or those that were found to be satisfactory and are in common use. The fungicides were vigorously shaken with the seed for a period of five minutes in stoppered Erlenmeyer flasks, after which the stoppers were removed, except in the case of Panogen 14 which remained in a stoppered Erlenmeyer for a period of 48 hours. Only Ceresan M slurry and Panogen 14 were applied in liquid form. A summary of the results is presented in Table XII.

TABLE XII

RELATIVE PERSISTENCE OF VARIOUS SEED PROTECTANTS ON VIABLE WHEAT, FLAX AND PEA SEED IN THE SOIL UNDER GREENHOUSE CONDITIONS

Treatment	Radius in mm. of Zone of Inhibition Around Treated and Untreated Seeds After Varying Periods in the Soil												
	Days in the Soil												
	Wheat					Flax			Peas				
	0	1	2	3	49	0	1	4	0	1	2	3	34
Ceresan M	8.3	3.8	0.0	0.0	0.0	7.5	0.0	0.0	17.0	3.5	0.8	0.5	0.0
Ceresan M Slurry	12.3	3.5	0.0	0.8	0.0	10.5	0.0	0.0	24.3	0.0	0.3	0.5	0.0
Panogen 14	10.3	5.0	2.5	0.0	0.0	9.3	0.0	0.0	7.5	0.0	0.0	0.0	0.0
C.I.L. Bunt Cure	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
MTH	6.8	1.0	0.0	0.0	0.0	1.5	0.0	0.0	16.3	0.0	0.0	0.0	0.0
Orthocide 406	15.0	15.5	11.3	12.0	3.3	18.3	13.0	12.0	18.8	25.0	25.0	17.8	10.5
Check	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

The fungicides appeared more persistent on wheat and pea seed than on flax seed in the soil. Orthocide 406 proved far superior to the other seed dressings in its ability to persist on each of the seed types in the soil. Even after remaining 49 days on wheat seed in the soil, it was capable of producing some inhibition of the growth of the test organism. Of the other seed dressings, Panogen 14 persisted longest on wheat (two days), Ceresan M dust, the longest on peas (three days), while none of the other fungicides persisted on flax seed even after one day in the soil. The hexachlorobenzene formulation on seed failed to show any fungistatic effect against the test organism. The wet seed treatments did not appear superior to the dry seed treatments, and except for Orthocide 406, the non-mercurial preparations proved inferior to the mercurial seed dressings in their ability to persist on seed in the soil. On wheat and peas, but not on flax, the Orthocide 406 preparation appeared to increase in fungicidal activity after one day in the soil. The ability of the various fungicides to adhere to wheat and flax seed when not exposed to the soil, exhibited the same trend, the order in decreasing adhesiveness being Orthocide 406, Ceresan M slurry, Panogen 14, Ceresan M dust, MTH, and C.I.L. Bunt Cure. While on pea seed Ceresan M slurry was the most adherent, Orthocide 406, Ceresan M dust and MTH were nearly equal; while Panogen 14 showed quite poor adhering qualities. No pronounced phytocidal effect from any of the treatments was noticed. Fungicide persistence tests on flax were dis-

continued after four days in the soil since the seeds had lost their seed coats by that time. They were discontinued on wheat and peas after 49 and 34 days in the soil, respectively, due to lack of treated seed material.

Discussion

Although the majority of the fungicides did not appear to persist on the seed for a prolonged period of time in the soil, it is possible that their protective effect may be exerted in the soil surrounding the seed for a longer period of time. Machacek (27) tested solutions of fungicides of various concentrations by absorbing them on paper disks and plating them on "seeded" agar. He found that the amount of diffusion of two of the fungicides was related to concentration, but that the diffusion of three fungicides was not so related. Thornberry (39) found that this method could not be used with cationic toxicants since they are adsorbed by the agar. Fungicides would also vary in their ability to act at a distance. Caution should therefore be exercised when evaluating the persistence of different fungicide preparations by the method employed in this paper.

Daines (7) found that in soils where mercurials are effective as fungicides, that the mercury compounds are reduced by the soil to metallic mercury which migrates in the soil as mercury vapors. Any factor that prevents the conversion of a mercury salt to metallic mercury destroys the fungicidal effects of the mercury. Some of these limiting factors are: The presence of mercury-precipitating ions; a

soil with a high mercury-binding capacity; also a soil having a high oxidation, and conversely, a low reducing potential. Under moist aerobic conditions, Booer (5) found that the addition of 0.2 percent powdered sulfur to the soil, whilst producing no immediate effect, completely eliminated in four to seven days the retardation of plant growth resulting from the addition of 0.02 percent mercury. The formation of mercuric sulfide is the means by which the toxic effect of mercury is eliminated from soil under field conditions from season to season (5).

Summary

Orthocide 406 proved far superior to the other seed dressings tested in its ability to persist on wheat, flax and pea seed in the soil. Of the other seed dressings, Panogen 14 persisted the longest on wheat, Ceresan M in dust form the longest on peas, while none persisted on flax seed in the soil long enough to make a comparison. Bunt Cure on seed proved inactive against the test organism used and hence its persistence on seed in the soil could not be measured. The wet seed treatments did not appear superior to the dry seed treatments, and except for Orthocide 406, the non-mercurial preparations proved inferior in their ability to persist on seed in the soil to the mercurial seed dressings. Although the ability of the various fungicides to adhere to wheat and flax seed when not exposed to the soil exhibited the same trend, they appeared more persistent

on wheat and pea seed than on flax seed in the soil.

SUMMARY OF THE PERSISTENCE OF CHEMICAL SEED PROTECTANTS ON SEED IN THE SOIL

Ceresan M, used as a dust, did not persist as well in the soil on non-viable wheat, flax and pea seed, as it did on the same types of viable seed. Regardless of treatment, the non-viable seed was more subject to decomposition in the soil than the viable seed. Of the six fungicide preparations tested, Orthocide 406 proved superior to the others in its ability to persist on viable wheat, flax and pea seed in the soil. The dry seed treatments were about equal to the wet seed treatments in their ability to persist on seed in the soil, and except for Orthocide 406, the non-mercurial preparations proved inferior to the mercurial seed dressings in this respect. According to this method of testing, the fungicides were more persistent on wheat and pea seed than on flax seed in the soil.

GENERAL DISCUSSION

The differences in the susceptibility to decay of the three kinds of seed included in this study, are probably explainable on the basis of the availability of their food supply to microorganisms of the soil. The garden pea seed which proved most susceptible to decay is known to have a readily available supply of simple carbohydrates together with a high protein content. This combined with its slower growth rate and its more vulnerable seed coat renders it very susceptible to decay.

More fungi than bacteria were found capable of causing seed decay of wheat, flax and pea seed in the soil. The fungi are likely capable of a faster entry into the seed whereas the bacteria would be more dependent on natural openings in the seed coat for their entry.

The methods employed for the isolation of organisms responsible for seed decay were satisfactory for obtaining these organisms from wheat and flax seed. They were unsatisfactory for obtaining them from pea seed, since a very low number of organisms isolated from decaying pea seed was separately capable of decaying them.

Soil environmental conditions exert a marked influence on the amount of seed decay, but usually only conditions

which are relatively less favorable to the host than to the pathogen result in much of this decay. The soil moisture and temperature when not at an optimum level for the host will have a strong bearing on the amount of seed decay. Soil low in moisture was found to favor the decay of wheat and flax seed, while high soil moisture favored that of pea seed. Soil temperature exerted its influence by encouraging the decay of flax and pea seed when low, and that of wheat seed when high.

In nature the amount of seed decay would vary with the changes in the soil environment. The critical period would be the first two weeks following seeding. Some control of the soil environmental factors may be obtained through cultural practices such as varying the date of seeding, rotating the crops and regulating the water supply. Wheat sown late in the spring was found to be much more susceptible to seed decay than that which was sown early. Flax seed is relatively susceptible to decay by fungi capable of decaying wheat or pea seed. This finding could apply in a crop rotation by avoiding the sowing of flax on land where much decay of wheat or pea seed had occurred.

Since the environmental conditions of the soil are relatively unpredictable, it is important to have at least the most susceptible seeds protected by chemicals. To be effective, the chemicals must inhibit the growth of the seed decay organisms on or near the seed in the soil. In addition,

ability to persist on the seed should increase their protective value. Of the six fungicides tested, only Orthocide 406 was outstanding in its ability to persist on seed in the soil, but aside from this fungicide, the non-mercurial preparations were inferior to the mercurial seed dressings in this respect. The majority of the fungicides only persisted on the seed in the soil for a day or two. Since many of them are known to be effective in reducing the incidence of seed decay, it may be that they exert their effect in the soil surrounding the seed for a longer period of time.

SUMMARY

1. Under all environmental conditions tested, pea seed showed the greatest tendency to decay in the soil, wheat the least, while flax was intermediate.

2. Decay of pea seeds was encouraged by high soil moisture (especially for the first three days after sowing) and by low soil temperatures.

3. High temperature and low moisture encouraged the decay of wheat seeds in the soil.

4. Low soil temperature and moisture favored preemergence blight of flax.

5. Fungi were found to be the most important seed-rotting microorganisms.

6. Seed held at low soil temperatures yielded most of the seed-rotting fungi which were isolated.

7. The number of fungal saprophytes isolated from decaying wheat seed increased directly with the period of time the seed was in the soil.

8. In general, the preemergence blighting fungi that were isolated from decaying wheat seed at soil temperatures of 15°, 20° and 30°C proved quite adaptable in

their ability to rot wheat seed over this range of temperatures.

9. Sterile soil with a high moisture content proved more conducive to wheat seed decay by fungi that had been isolated from rotting wheat seed than sterile soil with a lower moisture content.

10. Flax appeared to be more susceptible than wheat or peas to preemergence blighting by fungi that were isolated from decaying wheat, flax and pea seed.

11. Preemergence blighting of wheat by a fungus isolate was most severe when embryo-injured seed was used. Endosperm-injured seed proved only slightly more susceptible to it than normal seed.

12. The seed fungicide, Ceresan M, used as a dust, did not persist as well in the soil on non-viable wheat, flax and pea seed, as it did on viable seed of the same types.

13. Orthocide 406 proved much superior to the other fungicides tested in its ability to persist on viable wheat, flax and pea seed in the soil.

14. Except for Orthocide 406, the non-mercurial fungicides proved inferior to the mercurial seed dressings in their ability to persist on seed in the soil.

15. Most of the fungicides tested were more persistent on wheat and pea seed, than on flax seed in the soil.

A C K N O W L E D G M E N T S

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APPENDIX I

NUMERICAL DESIGNATION OF THE SEED DECAY MICROORGANISMS ISOLATED FROM ROTTING WHEAT, FLAX AND PEA SEED IN THE SOIL

(a) Fungi from Wheat Seed

15°C* 135$_{xx}$, 136$_{xx}$, 137$_{x}$, 138$_{x}$, 139$_{x}$, 310$_{x}$, 332$_{xx}$, 345$_{xx}$

20°C 114$_{xx}$, 127$_{xx}$, 143$_{xx}$, 145$_{x}$, 146$_{xx}$, 149$_{xx}$, 264$_{xx}$, 268$_{xx}$, 281$_{x}$, 286$_{xx}$

25°C 175$_{xx}$, 176$_{xx}$, 177$_{xx}$, 178$_{xx}$, 180$_{x}$, 181$_{xx}$, 184$_{xx}$, 191$_{xx}$, 192$_{xx}$, 194$_{xx}$, 195$_{xx}$, 204$_{xx}$, 216$_{xx}$, 219$_{x}$, 222$_{xx}$

30°C 159, 172, 240$_{xx}$, 247$_{x}$

(b) Bacterium from Wheat Seed

20°C 61$_{xx}$

(c) Fungi from Flax Seed

20°C F120$_{xx}$, F124$_{xx}$, F213$_{Rxx}$, F213$_{Gxx}$, F236$_{Rxx}$, F236$_{Gxx}$, P268$_{x}$**

(d) Fungus from Pea Seed

20°C P270$_{xx}$

x significant seed decay organism
xx highly significant seed decay organism
* temperature of the soil in which seeds were rotted
** Organism P268 was isolated from decaying pea seed, but was only found capable of significantly reducing the emergence of flax.